THE OUTCOME OF ACCIDENTAL BCG OVERDOSING DURING ROUTINE IMMUNIZATION OF NEONATES

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Thesis presented in fulfilment of the requirements for the degree of Master *of Medicine in the Faculty of Medicine and Health Sciences at Stellenbosch University*

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Declaration

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December 2019

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Abstract

In January 2015, 19 neonates were accidently given intradermal BCG culture SSI, a dose 62.5 times above the standard BCG dose for neonates at a Western Cape private hospital. After recognizing the error, all neonates who were given BCG culture instead of the BCG vaccine were identified and their parents informed. A panel of paediatric infectious disease specialists were consulted and the decision was made to start treatment with high dose isoniazid and rifampicin. Fourteen of the nineteen neonates were enrolled and followed-up in order to observe what proportion of neonates would develop adverse reactions. Complications that were observed for included severe local and regional adverse reactions and systemic BCG disease.

In this case series, no regional or systemic BCG disease occurred in any of the healthy term neonates who received an accidental overdose of BCG culture, instead of BCG vaccine. The conclusions of this case series are however limited by inconsistent follow-up and failure by the treating paediatricians to systematically record the required data.

Expected mild adverse reactions occurred in all the neonates (n=14, 100%) in our study, which was much higher than expected when compared to the usual occurrence of BCG adverse reactions to the Bacillus Calmette-Guerin (BCG) vaccine in neonates as demonstrated by the randomised control trial by Nissen et al. The more common occurrence of mild adverse reactions could be explained by the much higher dose of BCG administered. The majority of local adverse reactions were however of short duration with approximately one third resolving within 2 weeks and not a single one being present at 6 months.

Opsomming

In Januarie 2015 het 19 neonate by ’n Wes-Kaapse privaat hospitaal per abuis intradermale BCG-kultuur-SSI ontvang, ’n dosis 62,5 keer hoër as die standaarddosis BCG vir neonate. Nadat die fout ontdek is, is die neonate identifiseer en hul ouers ingelig. ‘n Paneel pediatriese infeksiesiekte spesialiste is genader en het besluit om met behandeling van hoë dosis isoniasied en rifampisien te begin. Veertien van die neëntien neonate is by die studie ingesluit en oor die loop van twee jaar opgevolg om vas te stel watter proporsie van neonate, wat gedurende die eerste week van lewe blootgestel is aan ‘n immunisasie met ‘n oordosis BCG kultuur, komplikasies sal ontwikkel. Spesifieke komplikasies waarvoor daar ge-observeer is het erge lokale en plaaslike reaksies asook sistemiese siekte ingesluit.

Geen plaaslike of sistemiese BCG siekte is in die gesonde term neonate wie blootgestel is aan ‘n oordosis BCG kultuur, in stede van BCG immunisasie, en ingesluit is by hierdie gevalle studie geobserveer nie. Die gevolgtrekkings van hierdie gevalle studie word egter beperk deur die sub optimale data versameling deur plaaslike pediaters asook die swak opvolg rekord van die neonate.

Die voorkoms van verwagte matige lokale reaksies in al die neonate (n=14, 100%) ingesluit in die gevalle studie was aansienlik hoër as die insidensie van lokale reaksies wat voorheen beskryf is om in neonate wat met BCG ingeënt is voor te kom. Die bevinding kan moontlik toegeskryf word aan die hoë dosis BCG wat toegedien is. Die matige lokale reaksies was van korte duur en het in ‘n derde van die groep binne twee weke opgeklaar met geen reaksies teenwoordig op ses maande opvolg nie.

Dedication

I dedicate this thesis to my husband, parents, sisters and parents-in-law as a tribute to their love, support and motivation

Acknowledgments

I am grateful to my supervisors, Dr JL Morrison and Prof M Cotton, who lead and supported me to complete my thesis.

I would like to acknowledge Doctors Van Niekerk, van Schalkwyk and Steenekamp, who assisted in obtaining consent on my behalf and granted me access to their records.

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List of abbreviations

AFB: Acid fast bacilli
ALT: Alanine transaminase
AST: Aspartate transaminase
BCG: Bacillus Calmette-Guerin
BMJ: British Medical Journal
CFU: Colony forming units
CRF: Case recording form
g/dL: Grams per deciliter
HIV: Human Immunodeficiency Virus
INH: Isoniazid
IQR: Interquartile range
LAD: Lymphadenopathy
MIC: Minimum inhibitory concentration
M. tuberculosis: *Mycobacterium tuberculosis*
SCID: Severe Combined Immunodeficiency
TB: Tuberculosis
RIF: Rifampicin
WHO: World Health Organization
U/L: Units per litre

1. Introduction

In January 2015 nineteen neonates received an accidental overdose of BCG during routine immunization at a private hospital in the Western Cape. Instead of receiving BCG vaccine, the neonates received BCG culture resulting in a 62.5-fold overdose. The paediatricians practicing at the private hospital approached the Department of Paediatrics and Child Health for advice on the management of the exposed neonates. From the resulting discussions, it was decided to treat the neonates with high dose isoniazid and rifampicin and to follow them up in a standardized method. This would enable those who advised the treating paediatricians to report on the outcome of these 19 neonates. It is the outcome of these 19 neonates that constitutes this thesis.

2. Literature review

Tuberculosis (TB) has been a cause of major human morbidity and mortality for millennia, but it was only during the endemic in Europe and North America during the 18th and 19th centuries that scientists were able to understand the pathology, which led to the prevention and cure of TB disease. [1]

It was during this time that two scientists, Albert Calmette, a specialist in tropical diseases, and Camille Guerin, a veterinarian, influenced by the work of Pasteur, who showed that repeated sub-culturing of rabies virus produced attenuated virulence, joined forces at the Pasteur Institute in Lille, to demonstrate that prolonged sub culturing would also decrease the virility of tubercle bacilli.[2] This discovery led to the attenuation of a virulent bovine strain of tubercle bacillus by sub culturing it in 3-weekly intervals, even during the German occupation of France during the First World War, until the vaccine was ready to be tested on humans 24 years later, in 1921.[3]

The first dose of *Mycobacterium bovis* Bacillus Calmette-Guerin (*M. bovis* BCG) was given orally to a neonate born to a mother with active pulmonary TB who died during childbirth.[4] The neonate, cared for by the grandmother and two siblings, all of whom were affected by TB, thrived. The positive outcome was followed by the mass production of BCG for medical use and between 1924 and 1928, 114 000 infants were vaccinated via the oral route without any serious adverse reactions occurring.[2]

The safety of oral BCG was only questioned when, in 1930 in the town of Lubeck, Germany, after vaccination of 251 neonates with oral BCG vaccine, 173 developed radiological and clinical signs of TB and 72 neonates died.[5] This first known BCG adverse event occurred due to the contamination of the vaccine by the virulent Kiel strain of *Mycobacterium tuberculosis* that was kept in the same refrigerator as the vaccine, but unfortunately tarnished the safety of BCG for a considerable period and contributed to the reserve with which the vaccine is administered in certain countries, even today.[6] An interesting finding after the careful clinical observation of the remaining 173 survivors, was their resilience after exposure to *M. bovis*.[5] An interesting observation in the group was that 68% of neonates who developed clinical disease after oral vaccination, achieved complete, spontaneous resolution of their symptoms despite the absence of anti-tuberculosis treatment.[5]

The sharp incline in TB cases after World War 2 and the ability to screen individuals with chest radiographs and tuberculin skin testing, created the need to improve vaccination coverage and gain a better understanding of vaccination efficacy.[1] This lead to the first large scale vaccination program sponsored by the United Nations Children's Fund and the Danish Red Cross under whose leadership 14 million people were vaccinated within 3 years. Large clinical trials were also established to test the safety and ability of different BCG vaccine strains to protect against TB in different populations and in different parts of the world.[3] A marked difference in efficacy between different strains and populations studied were already observed at the time. Although most studies showed at least some degree of protection against TB disease, it was clear that it markedly reduced the disseminated forms of TB.[7] However, great variation between individual efficacy studies were observed. These findings lead to the variation in usage of BCG vaccine between different countries in the world, ranging from routine use in endemic areas to targeted use in high risk populations only.[8]

The WHO estimated that in 2016, 1 million new cases of TB occurred in children and 253 000 deaths were caused by TB disease.[9] TB disease is notoriously difficult to diagnose in children and clinicians rely on a combination of non-specific testing and clinical evidence to support the diagnosis, as microscopy smear-positive for acid fast bacilli (AFB) cases only account for 0.5 – 3% of child TB cases annually.[10] The HIV endemic has also led to an increased vulnerability in children to develop TB disease - not just in the HIV-positive children, but also in HIV-negative children living with an HIV-positive adult. As history repeats itself we are again faced with a great need to effectively prevent TB disease in children and therefore BCG vaccine is used extensively with up to 100 million children receiving vaccination world-wide, annually.[6] Although the vaccine is considered safe in general, there are several complications associated with vaccination.

BCG is the only vaccine used where the aim of vaccination is to form an ulceration after administration.[6] A normal skin reaction will form an indurated area of approximately 5 – 15mm that will crust over and leave a 3 – 7mm scar after 6 – 10 weeks.[6] Other local and systemic adverse reactions of BCG beyond this local skin reaction has been well documented and described since the 1930's when the safety of the vaccine was first brought into question. Oral administration of BCG vaccine was the first suggested route by Calmette in 1921 as BCG was thought to be adequately absorbed in the gastrointestinal tract and into the lymphatic system where it would produce the delayed-type hypersensitivity reaction that

would ensure protection. This route has, however, been shown to be associated with higher rates of cervical lymphadenitis, suppurative lymphadenitis, otitis media and retropharyngeal abscesses - likely due to the higher dose of vaccine required to be administered. In 1940 Arlindo de Assis in Brazil administered over 2g of BCG orally in two adult volunteers over the 16-month course of the study with no subsequent detrimental health effects – this showed that an adult could tolerate doses far in excess of the doses routinely prescribed. Oral vaccination still remains one of the preferred routes of administration of BCG vaccine in Brazil, South America.[6]

Adverse reactions associated with the administration of BCG vaccine via the intradermal route occur at a rate of 4 - 30 per 1000 vaccinated neonates [6], are generally self-limiting and are limited to uncomplicated, regional lymphadenitis, abscesses and keloids. Local reactions associated with intradermal BCG are also postulated to be dose-dependent as was first reported after the Lubeck disaster in 1930. Here it was shown that a high dose of exposure to *M. tuberculosis* outweighed any natural resistance and would inevitably lead to disease and that disease severity was also directly related to the dose of exposure.[5] In 1968, a mass vaccination programme in Algiers showed a 50% decline in local adverse reactions after half the dose of BCG was used and in 1975 where a decrease in viable units of Danish BCG vaccine resulted in a reduction in lymphadenitis to an incidence of only 0,2 per 1000 by 1977.[6] Reports world-wide have shown a great variability in occurrence of adverse reactions with the only positive predictor of lymphadenitis being administration at an age younger than one month. Neonates are twice as likely to develop lymphadenitis than infants older than three months.[6] Injection technique and technical skills of the operator are also contributing factors to local reaction, occurring more commonly in areas where a change in injection from a subcutaneous to intradermal preparation was observed. Administration by inexperienced doctors versus experienced nurses have also shown an increase in adverse reaction occurrence.[6] Fatal dissemination occurs very rarely; one case in an estimated 0.19 – 1.56 per million vaccinations, and occurs exclusively in those with compromised immune systems. A great variability between adverse reactions associated with different strains of BCG vaccine exist. In neonates vaccinated at birth, the Danish-SSI 1331 strain is associated with higher incidences of suppurative lymphadenitis and abscesses. Alternatively, the BCG Tokyo and BCG Moscow strains have been associated with higher rates of osteitis. A British study in 2002 failed to prove a superior immunogenicity with BCG Danish-SSI 1331 despite

its increased teratogenicity and common occurrence of regional disease, when compared to BCG Glaxo, it's relative innocuous predecessor.[6]

Examples of regional disease:

- Subcutaneous abscesses and keloids
- Cutaneous lesions (Lupus Vulgaris and Scrofuloderma)
- Lymphadenitis

Examples of systemic disease:

- Osteitis/Osteomyelitis
- Dissemination of BCG

Regional disease such as suppurative lymphadenitis, abscess formation and ulceration occur commonly in healthy individuals, especially in areas where Danish BCG-SSI 1331 is used. The exact incidence is unknown but estimated to be between 4 – 30 per 1000 vaccinated neonates.[6][11] Two recent studies looking at the increased association between Danish BCG-SSI 1331 and suppurative lymphadenitis found an incidence of 0.1 per 1000 [12] and 0.4 per 1000 [13] immunizations in their respective study populations – still way below the expected average. Both suppurative and non-suppurative lymphadenitis can and should be managed expectantly. The addition of anti-tuberculosis treatment has been shown to only marginally reduce the duration of symptoms. Although widely practiced, needle aspiration and anti-tuberculosis treatment are only indicated in complicated or non-resolving cases, with surgical excision rarely required.[14] Cutaneous complications are less common and only a single case report of lupus vulgaris with scrofuloderma in a 3-year-old boy was identified in the recent literature.[15]

Systemic disease in children with normal immunity usually manifests as BCG osteomyelitis or osteitis. These children present with a limp or painful joint with decreased range of movement on the same side as vaccination between 6 – 12 months, and as late as 32 months after vaccination. Long bones are more commonly affected, but there have been reports of axial bones, such as the sternum, being involved. If an arthritis is present, the fluid is sterile with inflammatory markers improving after drainage, but symptoms remain persistent. Treatment requires multi-drug anti-tuberculosis treatment for 9-12 months depending on the clinical response.[16][17][18]

Dissemination of BCG infection is rare and severe disease occurs predominantly in children with significant T-cell related primary immune deficiency. In areas with a high HIV burden, dissemination occurs in HIV-positive neonates whose early diagnosis was missed and who did not access early antiretroviral therapy (ART). The dissemination is due to secondary immune deficiency.[19] A surveillance study conducted in South Africa detected 32 cases over a 3 year period, estimating the risk of disseminated BCG at 992 per 100 000 vaccinations in HIV-positive neonates.[20] Vaccination is further complicated by the asymptomatic nature of perinatal HIV transmission and the low sensitivity of the laboratory testing (HIV nucleic acid detection polymerase chain reaction) currently in use to detect HIV infection, in the first 48 hours of life.[21]

The occurrence of severe BCG adverse reactions, including disseminated BCG disease, is more prevalent in children with T-cell mediated immunodeficiency and specifically in Severe Combined Immunodeficiency (SCID). In a retrospective descriptive review of the complications of BCG administration in children with the diagnosis of SCID, a total of 349 children were vaccinated with BCG, of whom 258 (75%) were vaccinated in the first month of life. Of these children, 118/349 (34%) developed disseminated BCGosis with extra regional lymph nodes and lungs affected most commonly. The only significant differences between children who developed disseminated BCG disease and those who did not, were, regardless of vaccine strain or dose, the administration of vaccine within the first month of life, with a 2.03-fold higher prevalence of complications, and the total number of T-cells at the time of vaccination. A total number of 92 children demised in this study. The high complication and mortality rate can be attributed to the administration of vaccine prior to the diagnosis and absence of treatment of SCID.[22]

Disseminated BCG disease is usually treated aggressively with combined anti-tuberculosis treatment, but average survival is poor and it remains imperative to enquire about a family history of primary immune deficiency before administering the vaccine and to commence anti-retrovirals as soon as possible after birth if HIV-positive.[20]

There are various strains of BCG vaccine available. In South Africa, we currently use *M. bovis* BCG Danish strain 1331. The neonatal dose is 0.05 ml of the reconstituted vaccine. This is injected intra-dermally in the right deltoid area of the upper arm. This dose contains 1-4 x 10^5 colony forming units (CFU) of *M. bovis* BCG Danish strain 1331.

It is estimated that 80% of the organisms remain at the injection site for the initial 24 hours after administration, followed by local spread.[23]

Reports on BCG overdosing in neonates and children:

One of the first reports of BCG overdose dates from 1949 when, following a misunderstanding, 14 children reportedly received 750 times the usual dose of vaccine. A mild local reaction occurred within days to weeks and in two cases after a month. Local abscesses at the sight of injection occurred in all 14 children with draining sinuses complicating 5 cases and ulcerations erupting in the rest. Treatment was limited to topical ointment application, except in one case, where streptomycin was tried without success. Despite the absence of anti-tuberculosis treatment, the lesions all healed within 5 – 7 months.[24] In 1964 10 children between 3-13 years old were successfully treated with isoniazid (INH) for 60 days after an overdose of concentrated vaccine (12-15 times the normal dose). Isoniazid (INH) was used for the first time, after proving to reduce the bacterial load of TB in animal models. None of the children suffered any adverse reactions, including lymphadenopathy and local abscesses.[25] More recent reports of accidental overdose with BCG vaccine includes incorrect dosing due to incorrect reconstitution of the vaccine and a number of cases where percutaneous BCG was given intra-dermally resulting in a dose five times the upper limit of regular BCG administration. The dosing in the reported cases ranged from doses 5, 10, 15 and in one case, 20 times the suggested dose.[26] The reports vary in the outcomes of the exposed subjects.

In 1996, an article on BCG overdose at a British hospital in 1994 was published. In the report 556 children were followed-up after percutaneous BCG was administered intra-dermally at 5 times the regular dosage. Sixty-one children had local reactions including papules (n=6), lymphadenopathy (n=48), ulceration (n=6) and a single (n=1) subcutaneous abcess at the injection site that required fine needle aspiration. Of these children, only one child had lymphadenopathy >20mm in size and one child was treated with anti-tuberculosis treatment after being diagnosed with severe combined immune deficiency (SCID) at 6 weeks. No severe systemic BCG disease occurred.[27] The occurrence of local reactions was in keeping with those reported during the regular BCG dose immunization.[27]

In the same year an additional report was published on children who received subcutaneous BCG intradermally (five times the recommended dose) due to the similarity in the packaging of the two products. When comparing the group who had received the higher BCG dose to a group of children who received subcutaneous BCG at the regular dose, the size of the inflammatory induration was slightly larger and the duration of healing prolonged in the group who received the higher BCG dose.[28]

A retrospective review of vaccine misuse and overdose in France over a 4-year period prior to 2001, revealed 14 cases of suspected vaccine overdose in unknown quantities, all of whom experienced some form of local adverse reaction. The reactions were within normal limits seen during routine BCG vaccinations and therefore none of the cases received additional treatment.[29] In 2009, a 14-year-old had a surgical excision of an 8mm fluctuant lump and 6 weeks of dual anti-tuberculosis prophylaxis (rifampicin and isoniazid) after receiving 1ml instead of 0.1ml of BCG-Connaught vaccine. The excision was performed for a local reaction that, according to the WHO Guidelines [30] and a Cochrane review on the management of BCG adverse effects [31], was considered within expected limits of a local reaction.[23] Another large study reported the adverse reactions that afflicted 221 school going children who mistakenly received subcutaneous BCG instead of a tuberculin skin test (Tine test) at a single public school in Israel. Up to 160 (72.3%) of these children had a persistent local reaction at 120 days and 84 (38%) had an ulceration with discharge at the same time. Four children developed suppurative lymphadenitis of the axilla. Two of the four children suffering from suppurative lymphadenitis underwent surgical excision and drainage. All four were treated with isoniazid. After one year all children had complete resolution of adverse reactions.[32]

In 2012 a case report was published of a full-term neonate who received 1ml instead of 0.05ml of reconstituted BCG vaccine. The error was only discovered after 10 days and on examination at the time, no adverse reactions where documented. Isoniazid (INH) prophylaxis was continued for 6 months without further complications.[33]

A case report of regional BCG disease, by Al Maquabi et al, described a localized deltoid abscess in a neonate who received 20 times the normal vaccine dose. The abscess was aspirated and the neonate remained well without further treatment.[26] The subject of another unusual case report in the same year, was a preterm neonate who received 10 times the usual dose of BCG vaccine in the right arm. A prominent swollen area occurred on the right thigh and aspiration of the lesion revealed a sterile abscess. No mycobacterium was cultured. The child did not receive anti-tuberculosis treatment and at follow-up remained well.[34]

There is no definitive evidence for the treatment of BCG associated complications and adverse reactions. Aspiration of localized abscesses, surgical excision procedures and anti-tuberculosis treatment have been reported to be used with success.[23][26][35] A recent Cochrane systematic review reported no clear benefit in using a combination of anti-tuberculosis treatment (isoniazid, rifampicin or a combination of the two) when compared to expectant management.[31] This was especially the case for non-suppurative complications

where spontaneous resolution occurs between 4 – 6 months. In patients with suppurative lymphadenitis, needle aspiration is recommended while the instillation of isoniazid (INH) into the lesion requires further investigation.[31]

There is no standardized protocol for management of BCG overdose. Various approaches have been suggested including conservative observational management [13] and prophylaxis with isoniazid (INH) or a combination of rifampicin (RIF) and isoniazid (INH), depending on the resistance profile of the BCG strain used, for periods ranging between 6 weeks and 6 months.[33] All *M. bovis* BCG is resistant to pyrazinamide (PZA) and the Danish 1331 strain is also resistant to ethionamide (ETO), intermediary resistant to INH (minimum inhibitory concentration [MIC] 0.4mg/L) and susceptible to rifampicin (RIF; MIC 2.0mg/l), ethambutol (EMB; MIC 2.5mg/l) and streptomycin (SM; MIC 2mg/l).[36] Aspiration of abscesses with or without INH prophylaxis have been described and outcomes were equally favorable. In one case, where the error was recognized soon after the administration of the overdose, excision of the injection site was done and dual treatment with INH and RIF given for 6 weeks.[23]

Intravesical BCG for non-invasive bladder cancer is the most successful biological treatment for this cancer to date. It has been used therapeutically in adults with bladder carcinoma over the last 30-40 years.[37] BCG culture SSI contains BCG Danish strain 133, but at a much higher dose. One vial of BCG culture SSI typically contains $2.5x10^8$ CFU compared to the BCG vaccine dose 1- 4 x 10^5 CFU. The vial is reconstituted and instilled into the urinary tract where it triggers an immune response. The exact mechanism of action is still unknown, but the treatment has been shown to eradicate cancer cells, decreases disease recurrence and reduces the odds of disease progression significantly. The usual safety profile of intravesical BCG is favorable with systemic adverse reactions limited to case reports. Adverse reactions are estimated at 1% for local and 4.8% for systemic occurrence.[38] A retrospective analysis of 256 patients who received treatment over 6 years at a single institution, as well as an extensive literature search of cases with disseminated BCG after intravesical treatment since 1975, was published in 2014. No conclusive recommendations were made, but possible risk factors for dissemination such as traumatic instillation and underlying mucosal damage as present in underlying urinary tract infection and after transurethral resection, were identified. For this reason, the mean time delay between intravesical BCG therapy and transurethral resection is about thirty days in most centers. Immunosuppressed individuals and the elderly

have also previously been considered to have a higher chance of dissemination, but this theory has been revoked with large trials successfully including patients up to 80 years and immunocompromised individuals with equal success.[39]

Systemic disease after using this therapy in bladder cancer is well documented in several case reports with dissemination leading to pulmonary and hepatic granulomas, mycotic aneurysms and aseptic meningitis. No anti-mycobacterial regime is recommended for the treatment of dissemination of BCG following intra vesicular BCG. The prognosis is usually good with negligible mortality (5.4%) and long-term disability (7%).[39]

Cases of inadvertent intramuscular injection of BCG culture have also been reported. In one case an adult male was injected with 4 vials of BCG culture over a two-day period. He also received two intravesical administrations of BCG for bladder cancer in the preceding and following 2 weeks. The patient experienced initial acute fever, headache and pain at injection sites, but symptoms resolved spontaneously. It was only after the error was discovered that he was initiated on a 6-week INH/RIF prophylaxis course without any radiological or microbiological evidence of BCG dissemination. No further complications developed.[38] Pasteur and Hall also reported an adult who received an inadvertent intramuscular injection of BCG vaccine. This individual developed a severe and prolonged local reaction around the injection site and resolution was thought to be hastened by the administration of anti-tuberculosis treatment.[40] There have been no case reports in the literature of neonates or children receiving inadvertent intra-vesical BCG via the intramuscularly or intradermal route.

3. Scientific justification of the study

The discovery of BCG vaccination by, Albert Calmette and Camille Guerin, lead to large scale global vaccination campaigns and extensive research of BCG vaccine and its efficacy. After the Lubeck disaster in 1930 where BCG, contaminated by the virulent Kiel strain of mycobacterium tuberculosis, lead to 72 neonatal deaths, the safety of BCG was questioned. Vaccination with BCG is now largely considered to be safe although it is often associated with a mild local reaction. Rarely it can be complicated by severe regional and systemic disease and this is usually in children with immune deficiencies such as HIV and SCID.

Systemic BCG-osis is a rare severe illness that proves difficult to treat and commonly leads to death.[30]

The severity of systemic BCG-osis has led to continued concern surrounding BCG vaccine and especially in cases of inadvertent overdose as reported in the literature since its first occurrence in 1949 and more recently in 2015, when 19 neonates accidently received the largest reported overdose. The management of accidental BCG overdose has not been standardized. Various treatment options include a conservative approach observing for complication development, to treatment with anti-tuberculosis treatment and surgery of suppurative lymphadenopathy.[31]

Although BCG overdose is rare, the reporting of this case series remains important, especially in the light of anti-vaccination and media campaigns that strengthens mistrust of the public health system amongst the general public.[41]

This rare, but important overdose event as well as the scale of inadvertent BCG overdose could aid and inform management of future occurrences. Therefore, we intend to describe the management and outcome in the group of neonates who received a BCG overdose.

4. Research question

4.1 Primary objective:

What proportion of neonates accidently vaccinated, within the 1st week of life, with a high dose of BCG culture would develop local, regional and systemic adverse reactions?

4.2 Secondary objectives:

What proportion of neonates receiving high dose isoniazid and rifampicin would develop adverse drug reactions when being treated for an accidental overdose of BCG?

What proportion of neonates being managed for an accidental overdose of BCG would develop comorbid disease?

5. Material and methods

5.1 Context of the study:

In January 2015, 19 neonates were accidently given intradermal BCG culture SSI instead of BCG vaccine at a Western Cape private hospital with a well-established obstetric and paediatric service. BCG vaccine and BCG culture have similar packaging and were stored in the same refrigerator. BCG culture was administered to two sets of neonates within 7 days, after which the problem was identified.

BCG culture SSI was reconstituted and administered as recommended for the vaccine: Contents of a vial were reconstituted in 1 ml of saline and given intradermal at dosage of 0.05 ml. When reconstituted in the same way as BCG vaccine, 0.05ml contains 1.25 X 10^7 CFU, 62.5 times the standard BCG vaccine dose for neonates.

After recognizing the error, all neonates who were given BCG culture instead of the BCG vaccine were identified and their parents informed. Excision of the injection site was not a viable option, as the organisms are only localized at the site of the injection for 24 hours. After consulting several TB experts, the following care package was agreed on:

- Initial full blood count and differential count and HIV screening offered
- Immunological screening in the presence of suggestive family history of immune deficiency
- Ongoing clinical monitoring for adverse reactions (by local paediatricians)
- Preventive treatment with high-dose isoniazid (20mg/kg) plus rifampicin (20mg/kg) daily for six months
- Supplemental Vitamin B6 (pyridoxine)
- Monthly full blood counts to exclude lymphopaenia
- Monthly serum alanine aminotransferase (ALT) monitoring for drug induced hepatotoxicity resulting from the high dose anti-tuberculosis treatment

The rationale for anti-tuberculosis treatment was that a very high dose of BCG had been inadvertently given; approximately 62.5 x the regular dose. There was also a concern about the neonates being from a high endemic HIV area that could put them at risk of BCG dissemination as HIV status was not yet confirmed.[20]

The high dose of INH was based on the intermediate INH resistance pattern of Danish strain 1331 that can be overcome by doses of 20mg/kg and a MIC of.4mg/L.[36]

Complications that were observed for included severe local adverse reactions and systemic BCG disease.

5.2 Study population:

All the neonates (n=19) qualified to initially take part in the study.

i. Inclusion criteria:

a) Parents/legal guardian gave informed consent
b) Attended follow-up after the incident
c) Initiated on anti-tuberculosis treatment

ii. Exclusion criteria:

Although no neonates were purposefully excluded from our study, the following were excluded due to circumstances beyond the control of the study team,

d) Failure to obtain informed consent
e) Failure to obtain access to clinical records

5.3 Time frame:

The neonates were vaccinated in January 2015 and were followed up to 31 January 2017 for the purposes of this study.

5.4 Study description:

Prospective descriptive study

5.5 Data collection:

i. Description of data collection:

The neonates were followed-up by their treating paediatricians at their private practices. At the start of the follow-up period it was agreed that the paediatricians would collect data systematically for the study and conduct specified special investigations to monitor for the

possibility of adverse drug reactions. The treating paediatricians obtained informed consent from the parents prior to the start of the study. The high dose isoniazid (20mg/kg) and rifampicin (20mg/kg) for 6 months was initiated by the responsible paediatrician.

The neonates were followed up by the treating paediatrians. Initially they were followed up at monthly intervals for 6 months and then 6 monthly for 18 months. Any visits to the paediatricians for concomitant disease were recorded in the neonates' electronic database which was kept by the treating paediatrician.

The research doctor then collected the data from the treating paediatrician and entered the data onto a clinical recording form (CRF) that had a specific study number for each patient. The research doctor had no contact with the parents and could in no way influence the management of the affected neonates.

ii. Summary of data collected:

- Date of birth
- Date BCG administered
- Anthropometry at birth: weight, length, head circumference
- Weight at 6, 12, 18 and 24 months
- HIV status
- Immunizations received
- Descriptions and pictures of;
    a) Expected mild adverse reaction
    b) Regional disease: reactions from injection site, distinct from injection site and lymphadenitis
    c) Systemic disease: Osteitis or osteomyelitis
- Date of initiation of treatment [RIF/INH]
- Adherence to treatment
- Descriptions of drug related adverse reactions
- Brief notes on concomitant illness during follow-up

iii. Laboratory data collected:

Special investigations pertaining to the monitoring of adverse drug reactions over the 6 months of treatment and as agreed upon in advance by the parents and treating paediatricians,

were obtained from the laboratory by the study doctor. This included any full blood counts, liver function tests and renal function tests routinely performed. The results were entered on the CRF form.

5.6 Definitions of the outcomes:

i. Definitions of overdose:

For the purposes of this study a distinction between BCG vaccine and BCG culture is made:

a. BCG Vaccine:

A dose of *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) reconstituted to 0.5ml and administered intradermally, typically containing 1-4 x 10^5 colony forming units (CFU) of *M. bovis* BCG. The strain used was Danish BCG-SSI 1331.

b. BCG Culture:

A dose of *M. bovis* BCG used in the treatment of non-invasive bladder cancer and typically contains 2.5 x 10^8 CFU and 40 mg of sodium glutamate. The strain used is Danish BCG-SSI 1331.

ii. Definitions of observed adverse reactions:

The following reactions are described in keeping with the WHO criteria [30]:

a. Mild adverse reaction:

Injection site reaction characterized by a papule, it may be red, tender and indurated. The papule commences 2-3 weeks after vaccine administration and can continue to ulcerate for 2-5 months before leaving a superficial scar. Swelling of the ipsilateral regional lymph nodes (axillary, supraclavicular and cervical) may be present, will remain small <1.5cm and do not adhere to overlying skin.

b. Severe adverse reactions:

Two groups and are defined as follows:

1. Regional disease:

Injection site reactions:

- Injection ulcer: >10mm and persistent > 6 weeks [11]

- Subcutaneous abscess: local pain, tenderness, warmth and swelling
- Keloids: scar tissue that grows beyond the margins of the scar

Skin lesions distinct from the injection site:

- TB chancre: firm, shallow, non-tender ulcer with granulomatous base
- Lupus Vulgaris: small nodular lesions with gelatinous consistency
- Scrofuloderma: red/brown firm subcutaneous nodules develop into sinuses and tracts [42]

Lymphadenitis:

- Lymph node >20mm [11]

  Further divided into two groups,
- Adherent to overlying skin without suppuration[1]
- Adherent to overlying skin with suppuration;

2. Systemic disease:

Osteitis and Osteomyelitis

- Radiographic evidence of defect and cavity formation in the bone with abscess formation

Systemic Disease

- Systemic symptoms such as fever and weight loss plus two or more sites of infection beyond the site of vaccination, including lung, liver, spleen and/or bones

iii. Drug induced adverse reactions:

The Division of AIDS (DAIDS) grading of the severity of adult and pediatric adverse events 2017 [43] was used to classify drug induced adverse reactions.

a. Hepatic adverse reactions
   - Elevation of transaminases: Mild (1.25 – 2.5 x upper limit of normal), moderate (2.5-5.0 x upper limit of normal), severe (5.0-10.0 x upper limit of

[1]*Suppuration* refers to the presence of fluctuation on palpation, pus on aspiration or the presence of a sinus

normal) and life threatening (.10 x upper limit of normal) elevation of transaminases

- Clinical signs of hepatitis: jaundice, fever, vomiting, abdominal pain

b. Haematological adverse reactions

- Thrombocytopaenia: Mild (100, 000 – 125, 000/mL), moderate (50, 000 - 100, 000/mL), severe (25, 000 – 50, 000/mL) and life threatening (<25, 000/mL)
- Anaemia: Mild (8.5 – 9.9g/dL), moderate (7-8.5g/dL), severe (6-7g/dL) and life threatening (,6g/dL)

c. Renal adverse reactions

- Creatinine: Mild (1.1-1.3 x upper limit normal), moderate (.1.3 – 1.8 x upper limits of normal), severe (>1.8 – 3.5 x upper limits of normal) and life threatening (>3.5 x upper level of normal)

iv. General health effects:

a. Growth

General health was determined by serial anthropometric assessment over the follow-up period. Birth anthropometry and serial weights and heights were plotted on the standardized WHO child growth statistical distribution charts.

b. Other diseases

Concomitant illnesses during the follow-up period was noted.

5.7 Data management:

i. Data sources

The individual neonates' electronic medical records that were kept by the treating paediatrician.

Laboratory results from the laboratory data file pertaining only to screening tests for drug related adverse reactions.

ii. Data collection and data protection

A case recording form (CRF) was completed for each participant and they each received a unique identifying number. The CRF was then transcribed to an electronic data base (Microsoft Excel). All the personal identifiers were removed as soon as the patient's data was entered onto the electronic database. The electronic data was saved and backed up on a secure password protected personal computer and an encrypted external hard drive to which only the researcher and the supervisors had access.
All paper documents were stored in an allocated space in the secure storage cabinet for research files in the Department of Paediatrics and Child Health.

5.8 Data Analysis:

The basic features of the data were described using descriptive analysis. Quantitative descriptions where made in order to summarize the data and univariate analysis was used to better describe the variables. The frequency and central tendency of distribution was calculated with use of median values and interquartile range (IQR).

5.9 Ethics:

Permission was granted to do the study by the Human Research Ethics Committee. The ethics committee file number is: S16/10/234. A copy of the ethics certificate is included as appendix A to the thesis.

6. Results:

6.1 General

The sample consisted of 19 neonates who received vaccination with BCG culture instead of BCG vaccine within the first 4 days of life. The number of days between inadvertent vaccination and initial assessment ranged from 7-12 days with the median being 7 days (IQR 6 - 11). Of the 19 neonates, data was available for 14 (74%) neonates as one of the paediatricians was reluctant to give the access to the data of 5 of the neonates. The sample (n=14) included healthy term neonates with 11 (79%) neonates plotting around the median, 2 (14%) plotting on the -2 Z score, and 1 (7%) that weighed above the +3 Z score of weight for

age. The median birth weight was 3.26kg (IQR 3.1 – 3.6). All neonates included were found to be documented HIV unexposed or HIV negative.

6.2 Adverse reactions resulting from the BCG overdose:

i. Adverse reactions at first visit and at 2-week follow-up:

A local injection site reaction leading to a papule that was either discoloured or not, was experienced by all neonates (n=14, 100%) on the first visit within the first two weeks after BCG administration. The size of the papule was greater than 5mm in 8/14 (58%) neonates. This was accompanied by ipsilateral lymphadenopathy (<10mm) in 4/14 (29%) and a superficial ulcer (<10 mm) in 2 /14 (14%) cases. (see Table 1)

ii. Adverse reactions at 2-week follow-up:

At the 2 weeks follow-up, the proportion with a visible papule had decreased to 8/14 (57%), ipsilateral lymphadenopathy 6/14 (43%) and an injection site ulcer to 1/14 (7%).

Adverse reactions present at visit:	**1st Visit**	**2 weeks**	**8 weeks**	**16 weeks**	**6 months**
Injection site reaction:					
Papule, with or without discoloration	14	8	5	5	-
<5mm	6	2	5	5	-
>5mm	8	6	-	-	-
Lymphadenopathy <10mm	4	6	2	2	-
Injection site ulcer <10mm	2	1	4	-	-
Persistent >6weeks	-	-	4	-	-
Superficial scar	-	-	-	9	14
Regional disease:	-	-	-	-	-
Systemic disease:	-	-	-	-	-

Table 1 The adverse reactions, regional and systemic complications are presented. The data for months 12,18 and 24 is not included as they did not differ from the effects and complications at 6 months.

iii. Adverse reactions at 8 weeks, 16 weeks and 6 months.

All the adverse reactions had decreased during the follow-up period. (see Table 1). At the 6-month follow-up there were no remaining adverse reactions. All 14/14 (100%) participants had a superficial scar at the site of the immunization.

a. Injection site reactions:

Six participants (6/14; 42%) had recorded eruption with oozing ulceration. The commonest time period that this occurred was at 8 weeks (4/6; 67%) Not one of the cases met the definition of severe adverse reaction as none had ulceration >10mm plus persistent oozing >6 weeks. The ulceration in 4/6 (66%) continued draining between 12-14 weeks before resolution.

b. Subcutaneous abscess:

Although papule formation, draining and oozing occurred, local abscess with pain, warmth and swelling was not recorded in any neonate.

c. Keloids:

No Keloids were reported. All scars were <10x10mm.

No TB chancre, Lupus Vulgaris or Scrofuloderma were reported.

d. Lymphadenitis

No lymph nodes >20mm where reported.

e. Systemic disease:

No systemic dissemination of BCG, including osteitis and osteomyelitis where recorded. We acknowledge, however that the occurrence of bone-related systemic complications can occur outside of the two-year follow-up that was conducted in this study.

6.3 Adverse drug reactions:

All 14 (100%) of the neonates received rifampicin 20mg/kg/day and high-dose isoniazid 20mg/kg/day.

Treatment was initiated on the first day of initial assessment, within 7 -12 days after the vaccination with the median being 7 days (IQR 6 - 11), post vaccination. There were no interruptions to treatment and all participants were adherent to treatment.

As part of the care package, paediatricians also agreed to prescribe prophylactic pyridoxine, but in 3 participants (3/14; 21%) prophylaxis was terminated within the first month due to increased observed cramping after administration resulting in parental anxiety.

Treatment was well tolerated and parental anxiety due to orange discoloration of bodily fluids were noted.

i. Hepatic adverse reactions

Serum aspartate transaminase (AST) levels were performed in 12/14 (86%) participants on two occasions and one (1/14; 7%) had a single test at 4 months. No one had serum alanine transaminase level performed as agreed to prior to the observation period. The elevation of AST >60U/L seen in 2/14 (16%) of the neonates is 2.5 to < 5.0 x upper limits of normal. This is considered a moderate adverse reaction according to the Division of AIDS grading the Severity of Adult and Pediatric Adverse Events 2017 [43]

No participant had recorded clinical features suggestive of hepatitis, including jaundice, fever, vomiting or abdominal pain.

ii. Hematological adverse reactions

Six (6/14; 43%) neonates had a baseline full blood count performed at the first visit with only one (1/14; 16%) having a follow-up full blood count. In 4/6 (67%) of the full blood counts performed, the full blood counts were within the range of normality. An interesting finding was that of a thrombocytosis >700 000 x 10^9/l, accompanied by a monocytosis with an absolute monocyte count >1.3 x 10^9/l at baseline full blood count in 2/6 (33%) of the neonates. These findings are consistent with an acute phase reaction response. Similarly, the single follow-up full blood count was within the normal range.

iii. Renal adverse reactions

No renal function testing was done on any of the neonates.

6.4 General growth and development.

Follow-up visits were well attended with all 14 (100%) participants visiting once a month for the first six months and thus completing treatment under supervision. All participants were immunized according to the national programme for immunizations. Only 6 (6/14; 42%) weights were recorded at the six-month visit and all were growing above the median for age. No heights were recorded and no head circumferences were done. Although no objective measurements were done the remaining 8/14 (57%) participants were reported by the treating paediatrician as growing well.

At 12 months only eight (8/14; 57%) participants were seen at follow-up; with only four (4/8; 50%) weighed. Two (2/4; 50%) were growing above the median and two (2/4; 50%) below the median for age with growth plateauing slightly. All attendees were noted to be immunized according to national guidelines and subjectively the paediatricians noted that the participants were growing well and developing normally.

At 18 months, none (9/14; 64%) attended follow-up; only four (4/9; 44%) weights recorded all of which were above the median for age.

At 24 months ten (10/14; 71%) participants attended follow-up and only five (5/10; 50%) weights were done, all of which were above the median for age. Once more all attendees were noted to be immunized, growing well and developing normally.

There were no hospitalizations.

One participant had a proven urinary tract infection and had low levels of serum Immunoglobulin A (IgA). This persisted when followed-up. Another child was screened for IgA deficiency as a sibling already had the diagnosis, but had normal serum IgA levels.

7. Discussion

In this case series, no regional or systemic BCG disease occurred in any of the healthy term neonates who received an accidental overdose of BCG culture, at a dose 62.5X above the standard BCG immunization dosage for neonates, instead of BCG vaccine. All of the children had mild adverse reactions as per the defined parameters of WHO criteria.[30] The conclusions of this case series are however limited by inconsistent follow-up and failure by the treating paediatricians to systematically record the required data.

Expected mild adverse reactions occurred in all the children (n=14; 100%) included in our study, which was much higher than expected when compared to the usual occurrence of BCG adverse reactions to the Bacillus Calmette-Guerin (BCG) vaccine in neonates, as evaluated in a randomized controlled trial by Nissen, et al.[44] The more common occurrence of the mild adverse reactions could be explained by the much higher dose of BCG administered. The majority of the local adverse reactions were however of short duration with approximately one third resolving within 2 weeks and not a single one being present at 6 months.

When compared to a study of 556 children who received 5 times the upper limit of normal BCG vaccine [28], only 11% of children in this large group of infants developed any local

adverse effects with 8.6% having lymphadenopathy and one child having a lymph node >20mm. In our group, 42% of children had palpable lymph nodes, but none had lymphadenopathy >20mm. Ulceration was common in both studies with 1% having ulcers larger than 10mm, but not draining for more than 6 weeks in the BMJ study, compared to the 42% in our study whose ulcers where consistently <10mm, but drained for up to 14 weeks.[28]

In the retrospective review of spontaneous reported vaccine misuse and overdose in France, only 14 reports of possible BCG overdose were identified, three of which included both misuse and overdose.[29] In this study, of equal size to our study, all subjects experienced mild adverse reactions. No severe adverse reactions were reported and none of the children received any form of treatment. The exact amount of BCG administered was unfortunately not recorded making a comparison difficult.[29]

Study:	**UK 1996**	**Israel 2001**	**Middle East 2012-2015**	**Australia 2009**	**Cape Town 2015**
Overdose:	5x Standard dose	5x Standard dose	10-20x Dose	10x Standard dose	62.5x Standard dose
Children affected (n):	**556**	**226**	**2**	**1**	**14**
Mild adverse effects					
Papule	-	-	1	-	14
Ulceration <10mm	48	58	-	-	6
Lymphadenopathy <1.5mm	-	-	1	-	6
Regional adverse effects					
Ulcer >10mm, >6/52	1	-	-	-	-
Abscess	1	-	1	1	-
Lymphadenopathy >20mm	-	-	-	-	-
With suppuration +	-	4	-	-	-

Table 2 Comparison of findings reported in studies describing accidental BCG overdose.

A study conducted in Israel [32] where 226 school-going children received BCG vaccine subcutaneously instead of TST, and at ten times the dose, 62% had a local reaction on day 18 after vaccination and 72% on day 120. Of these children, 26.6% had ulceration that drained

continuously for 16 weeks. The only children treated with anti-tuberculosis treatment were four children who developed suppurative lymphadenitis of which two required surgical drainage. The anti-tuberculosis treatment was initiated after the development of the complications. There are significant differences between the neonates in our study sample and this group that prevents a direct comparison of results. Keeping the heterogeneity of the two groups in mind, the high occurrence of expected reactions and ulceration is in keeping with our study, but the development of lymphadenitis was not seen in our sample despite the high dose of BCG and young age that should have theoretically contributed to the development of a higher proportion of lymphadenitis. The absence of lymphadenitis in our study could possibly be attributed to the anti-tuberculosis treatment received.

Adverse reactions secondary to BCG vaccination and/or overdose is well recorded in the Middle East. An investigation of 34 children with possible disseminated BCG disease was reported in 2015.[45] Three varying case reports from 2012 – 2015 were found, describing neonates who received a BCG overdose of 10 and 20 times the maximum dose.[26][33] In the case report a neonate received a BCG overdose of 20x the regular dose of vaccine. No adverse reactions were recorded within the first 10 days following the overdose and none were recorded after the completion of isoniazid chemoprophylaxis for 6 months. In a separate case, a 3-4cm lump with no lymphadenopathy was described following a BCG dose of 20 times the usual dose. No prophylaxis was initiated. The lump resulted in an abscess in the deltoid muscle (2.2x1.5cm) within the first month. After aspiration of 2ml sterile pus, no further treatment was given and no further complications recorded by 18 months.[26] In an unusual case, a preterm infant developed an abscess in the right thigh, 5 months after administration of 10x normal BCG vaccine. The abscess was sterile, and although the cause was never proven it was speculated to be secondary to BCG administration. It is this case that some authors use to justify the treatment of neonates and children who accidentally receive a BCG overdose as the authors argue that severe regional adverse reactions are more common in this group who had accidentally received a BCG overdose.[34]

A case report illustrating the development of an unexpected local adverse reaction with BCG overdose is that of a 14-year-old girl who received Connaught vaccine ten times the usual dose, who developed a subcutaneous fluctuating mass of 8mm with no lymphadenopathy within a few hours. The mass was surgically excised within 12 hours and she received prophylaxis with RIF/INH for 6 weeks.[23] This aggressive management of a local adverse

reaction is not supported by any other data in the literature. No overdose up to date has been associated with systemic BCG disease and the rationale behind this aggressive management is questionable. As the Cochrane review suggested [31], simple fine needle aspiration might have been sufficient, less traumatic and likely resulted in a similarly good outcome.[26] Due to the delay in recognition of the error in our study, as well as a lack in supporting evidence suggesting aggressive surgical management, surgical excision was not performed.

Prophylaxis and/or treatment used in the management of BCG overdose is diverse and the specific anti-tuberculosis treatment used, dependent on the strain of BCG implicated. Of the cases reviewed in this study, six of the eight reports gave no routine treatment after overdose.[26][27][29][32][34] In one case, treatment was only given after complications occurred [32], and in two cases prophylaxis was initiated.[23][33] The rare occurrence of serious local adverse reactions in the untreated groups, as well as the absence of systemic dissemination could motivate for a more conservative approach to BCG overdose with careful serial follow-up and targeted management of adverse reactions as they occur. On the other hand, the serious local adverse reactions that were reported; the child with lymphadenopathy >20mm in the BMJ study who received a dose 10x the normal dose [27], the 4 children with suppurative lymphadenitis in the Israeli study (5x normal dose) [32] and the 2 case reports of abscesses in the Middle East, after overdoses 10 and 20 times the normal dose respectively, all occurred in children who did not receive prophylaxis.[26][34] These reactions could alternatively be argued to be mild and might not have been treated if they occurred after routine vaccination.[46] If treatment of these complications under regular conditions were indicated, fine needle aspiration would have been the treatment of choice and the addition of chemoprophylaxis physician dependent.[31] If the decision to add anti-tuberculosis treatment is indicated, most will regard monotherapy such as INH sufficient, however this would only be appropriate for vaccine containing BCG strains susceptible to INH.[36] The surgical excision of the injection site previously described, would not be recommended when considered retrospectively.[23]

In our case series, the neonates involved received a six-month course of RIF and high dose INH. The rationale for 6-month treatment was the extent of over-dosage with 62.5x the regular dose, a dose which was not previously described in neonates or children. It is known that young children, particularly neonates are more susceptible to the development of TB [10] and that young infants' immune systems are immature making them more susceptible to systemic complications. There was also a concern about the neonates being from a high

endemic HIV area that put them at risk of BCG dissemination prior to the HIV status being confirmed.[20]

The high dose of INH was based on the intermediate resistance pattern of Danish strain 1331 that requires higher doses of INH to achieve the required MIC.[36] Drug-induced adverse reactions were expected in our study due to the high dose of INH used. Mildly elevated AST levels were observed in two of the neonates, but no clinical observation of severe adverse reactions were, however, reported. Accurate reporting was limited by the non-adherence of the paediatricians to follow the protocol to detect the adverse drug reactions. This was further complicated by the subjective assessment of drug adherence.

We acknowledge the following limitations of our study. The study was complicated by difficult access to records, poor follow-up and inadequate data capturing at follow-up visits. These limitations occurred in spite of the paediatricians agreeing prior to commencement of the study to the follow-up procedures. The small population affected and especially the small sample size recruited affects the validity of the study. The biochemistry as agreed on in the initial care package was not adhered to and the possibility of mild adverse drug reactions cannot be excluded. No participant however experienced any clinical symptoms related to drug induced reactions. The duration of follow-up for 2 years does not completely exclude long term complications such as, osteitis and osteomyelitis, which can occur up to 32 months following BCG vaccination, but the strain used in this case, Danish BCG-SSI 1331, is not known to be associated with these complications.[6] In addition not all 14 neonates attended follow up for the full 24 months. Finally, the study was not a randomized study with all the neonates receiving treatment. The small proportion of local adverse reactions and the absence of regional and systemic complications cannot solely be ascribed to the use of treatment.
Multiple challenges were faced during the conduction of this study. The biggest contributor being that the overdosing occurred in a privately funded medical care setting with physicians and managers alike, more interested in the possibility of liability and litigation than medical research. Scrutiny by the media blew the entire situation out of proportion and angered the parents involved. As an investigation into the matter was already under way during the initiation of our study, this directly impacted our relationship with the stake-holders involved and limited our ability to access records freely. In the light of litigation, research and scientific reporting was certainly not viewed as a priority. Obtaining informed consent was a

challenge due to the afore mentioned complications. This mismatch between the priorities of the hospital, the treating clinicians and the investigators impacted on the quality of the study.

8. Conclusion

No systemic BCG disease has ever been reported in any case of inadvertent injected BCG overdose in the literature, regardless of dose, strain of BCG, age of the affected individual, or treatment administered.

The findings of our study are in keeping with historic and recent literature that suggest that although minimal expected local adverse reactions will occur with an overdose of BCG, severe complications are rare and possibly limited to children not receiving treatment. Systemic dissemination is extremely rare, undocumented in the context of overdose and only relevant in children who are at risk of dissemination due to inherited primary immunodeficiency or acquired immunodeficiency such as HIV. It is important to note that in these children dissemination will occur with BCG administration, regardless of dosage. We therefore recommend screening for these conditions prior to administration of BCG and certainly as a matter of urgency, after the possibility of an overdose.[45]

In the event of adverse BCG administration possible treatment options reported includes a conservative approach with careful serial follow-up and targeted management with a combination of anti-tuberculosis treatment (in an appropriate combination and dose relative to the strain injected) and fine needle aspiration of abscesses and suppurative lymphadenitis as they occur. An alternative management would be prophylaxis for a short duration of 6 weeks to 3 months, keeping in mind that treatment of adverse reactions will only shorten the duration of symptoms marginally.

9. Recommendations

i. The ideal alternative to the treatment of BCG overdose would be to prevent it from occurring altogether. We know that overdosage is the result of multiple factors including equipment failure, such as the size and type of syringe used, technique of intradermal injection rather than subcutaneous injection, as well as similarities in packaging between different strengths of BCG, making it easy to mistake a more

concentrated vial of culture for the less concentrated vaccine. In our study, it was the similarity of packaging between the BCG vaccine and BCG culture that lead to the inadvertent injection of the neonates. In four of the eight reported studies, it was also the similarity between intradermal and subcutaneous BCG products that lead to an inadvertent overdose. This highlights the need for pharmaceutical companies to use distinct dissimilar packaging that is clearly marked as a preventative measure in the inadvertent overdose of BCG. In addition it would be prudent to store the different BCG products separately.

ii. No definitive management recommendations can unfortunately be made due to the heterogeneity of the literature review and the poor external validity of our specific case series. We do, however suggest that a conservative approach with careful serial follow-up and targeted management of complications with a combination of anti-tuberculosis treatment and fine needle aspiration of abscesses and suppurative lymphadenitis as they occur is a viable treatment option. An alternative management would be prophylactic chemoprophylaxis for a short duration of 6 weeks to 3 months, keeping in mind that treatment of adverse reactions will only shorten the duration of symptoms marginally. Due to the mild adverse reactions experienced in this review of the literature, the aggressive surgical excision of injection sites cannot be advocated for at this time.

10. Future research

The impacts that the privatization of medical care, the rise of an era of medical litigation and the media has on scientific reporting [41] should be explored further. In this era of access to unlimited information and biased opinions, the medical scientific community should send a clear message that supports and furthers the knowledge of our colleagues and peers and also provides recommendations for management. This includes reporting and recommendations based on clinical evidence reviewed, rather than expert opinion alone as opinion can lead to dangerous assumptions that are wrong and can further the general public's mistrust of vital components of the public health system.

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12. Appendices

APPENDIX A: ETHICS CERTIFICATE

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot • your knowledge partner

02-Dec-2016 Greybe, Leonore L

Ethics Reference #: S16/10/234

Title:

A DESCRIPTIVE CASE SERIES OF THE OUTCOME OF ACCIDENTAL OVERDOSING OF NEWBORN INFANTS WITH BCG CULTURE INSTEAD OF BCG VACCINE

Approved with Stipulations New Application

Dear Dr, Leonore Greybe,

The **New Application** received on **26-Oct-2016**, was reviewed by members of **Health Research Ethics Committee 1** via Expedited review procedures on **30-Nov-2016**.

Please note the following information about your approved research protocol:

Protocol Approval Period: **02-Dec-2016 -01-Dec-2017**

The Stipulations of your ethics approval are as follows:

The investigators to confirm that the incident and finding were or are going to be reported to the Pharmacovigilance committee of the Medicines Control Council.

Please remember to use your **protocol number** (**S16/10/234**) on any documents or correspondence with the HREC concerning your research protocol. Please note that the

HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or

monitor the conduct of your research and the consent process.

After Ethical Review:

Please note a template of the progress report is obtainable on www.sun.ac.za/rds and should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit. Translation of the consent document to the language applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372 Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms. Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Helene Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research. For standard HREC forms and

documents please visit: www.sun.ac.za/rds

If you have any questions or need further assistance, please contact the HREC office at.

Included Documents:

MMED Protocol version 3 Final.pdf CV Morrison J.doc Investigator Declaration - Prof M Cotton.pdf Appendix B - Data Collection Tool.pdf Checklist.pdf

Appendix D - Budget.pdf Appendix A - Researcher CV.pdf CV M Cotton.pdf Appendix C1 - Consent Form.pdf Investigator Declaration - Dr J Morrison.pdf Investigator Declaration - Dr L Greybe.pdf Appendix C2 - Information leaflet.pdf HREC.pdf

Sincerely,

Franklin Weber HREC Coordinator Health Research Ethics Committee 1

APPENDIX B: CASE REPORT FORM

Case Report Form

Demographic data

Identifier no:
Gender:
Date of birth:
Birth anthropometry: Weight (kg – 2nd decimal place):
Length (cm):
Head Circumference (cm):

Weight at 6 months (window: 20-26 weeks) (kg)_____
Wt for age_____
Weight at 12 months (window: 50-54 weeks) (kg)_____
Wt for age_____
Can add for 18 and 24 months

Date that BCG was given:

Age of child (days) at time BCG given:

Prophylaxis - **Date initiated:**

Date completed:

Drugs given:

Any interruptions or side effects:

HIV status: Positive

Negative

Exposed

Unknown

If positive then:

CD4_____(date)_____

Viral Load_____(date)_____

HAART

If yes: duration

Any other immune concerns:

BCG –Local reactions: yes/no

If yes: describe fully with date of occurrence and clinical course

BCG – Systemic Reactions: yes/no

If yes: describe fully with date of occurrence and clinical course

Other major clinical events from birth/ hospital admissions:

Blood results:

FBC:

Date and results:

LFT:

Date and results:

APPENDIX C: TABLES

Table 1. Occurrences of adverse effects described at each follow-up visit:

Adverse reactions present at visit:	**1st Visit**	**2 weeks**	**8 weeks**	**16 weeks**	**6 months**
Injection site reaction:					
Papule, with or without discoloration	14	8	5	5	-
<5mm	6	2	5	5	-
>5mm	8	6	-	-	-
Lymphadenopathy <10mm	4	6	2	2	-
Injection site ulcer <10mm	2	1	4	-	-
Persistent >6weeks	-	-	4	-	-
Superficial scar	-	-	-	9	14
Regional disease:	-	-	-	-	-
Systemic disease:	-	-	-	-	-

Table 1 The adverse reactions, regional and systemic complications are presented. The data for months 12,18 and 24 is not included as they did not differ from the effects and complications at 6 months.

Table 2. Comparison of findings between studies reporting on BCG overdosing:

Study:	**UK 1996**	**Israel 2001**	**Middle East 2012-2015**	**Australia 2009**	**Cape Town 2015**
Overdose:	5x Standard dose	5x Standard dose	10-20x Dose	10x Standard dose	62.5x Standard dose
Children affected (n):	**556**	**226**	**2**	**1**	**14**
Mild adverse effects					
Papule	-	-	1	-	14
Ulceration <10mm	48	58	-	-	6
Lymphadenopathy <1.5mm	-	-	1	-	6
Regional adverse effects					
Ulcer >10mm, >6/52	1	-	-	-	-
Abscess	1	-	1	1	-
Lymphadenopathy >20mm	-	-	-	-	-
With suppuration +	-	4	-	-	-

Table 2 Comparison of findings reported in studies describing accidental BCG overdose.